PATENT
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Lisa

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Jinny Nguyen

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

Jacob Sten PETERSEN

Serial No.: 09/064,682

Filing Date: April 22, 1998

For: COMBINATIONS OF ANTIGEN AND MUCOSAL BINDING COMPONENT FOR INDUCING SPECIFIC IMMUNOLOGICAL TOLERANCE

Examiner: R. Swertz

Group Art Unit: 1641

DECLARATION OF JACOB STEN PETERSEN PURSUANT TO 37 C.F.R § 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

I, Jacob Sten PETERSEN, declare as follows:

1. I am an inventor of the above-referenced patent application, and am familiar with the contents thereof.

2. I currently reside at 5501 17h Avenue NE, Seattle, WA 98105.

3. I have conducted experiments to demonstrate that the GM1 binding activity of cholera toxin B (CTB) is responsible for a stronger induction of oral tolerance against a given

antigen, when the antigen is administered in combination with CTB. This method of administration is similar to what is described in the Specification of the above-referenced patent application.

4. In the LCMV model, as described in Von Herrath MG et al. in Journal of Clinical Investigation, 98(6), 1324-1331 (1996) a copy of which is enclosed, the initiating self-antigen is a viral protein expressed as a transgene in the beta cells of the islets of Langerhans by a rat insulin promoter. Type 1 diabetes develops in all mice after initiation by viral (LCMV) infection and the T cells and cytokines involved in causing disease are known. In brief, the transgenic mouse line expresses the nucleoprotein (NP) of LCMV. Peripheral ignorance to the viral (self) transgene is broken by challenge with the LCMV, and diabetes develops in >95% of such mice.

5. The experiments were carried out as follows. Diabetes incidences were recorded 4 weeks after LCMV infection. Diabetes is defined by blood glucose levels of greater than 350 mg/dl. Animals were fed twice a week beginning two weeks before LCMV infection and feeding was continued for 4 weeks, twice a week after virus infection. 8-10 animals per group were tested. Mixture refers to 10 μg CTB mixed with 100 μg of insulin (not conjugated). The mixture was preincubated with GM1 (10 μg, Sigma) for a minimum of 30 minutes, before feeding in order to allow binding to CTB.

6. The following results were obtained from the experiments. 10 μg CTB and 100 μg insulin mixture/dose can prevent the development of diabetes since only 40% (4 out of 10) of the treated animals developed diabetes 4 weeks after LCMV infection compared to 90% (9 out of 10) of sham treated animals. If CTB was preincubated with GM1 in order to prevent binding to the GM1 receptor in the gut prior to feeding the mixture to the animals, then the effect of CTB is inhibited since 63% (5 out of 8) of the animals develop diabetes.

7. These date clearly demonstrate that binding of CTB to GM1 is very important in order to potentiate oral tolerance using CTB. It is my opinion that a person skilled in the art will clearly be guided to consider that other molecules/proteins with similar GM1 binding activity, e.g., LTB, will have the same effect, especially since LTB binds GM1 with an affinity that is equal to the affinity of CTB of GM1.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

November 8, 2000
Date

Jacob Sten PETERSEN